X-ray reflectivity study of monolayers of amphiphilics at the air–water interface

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Abstract. Monolayers of amphiphilic molecules spread at the air–water interface have been studied using x-ray reflectivity, a technique which allows an independent determination of their thicknesses and densities (i.e. structural parameters), as well as their roughnesses (due to thermally excited capillary waves). The phase diagrams of C_{15}, C_{21} and C_{29} fatty acids and of the phospholipid L-α-dipalmitoylphosphatidylcholine (DPPC) have been investigated and their phase transitions characterized. Evidence for the liquid-expanded–liquid-condensed transition is given by an abrupt increase in the thickness of the aliphatic medium, and the structure is characteristic of the liquid-condensed phase below and above the triple point. A strong decrease in the roughness is observed at the transition to the solid state. This striking feature is attributed to the bending rigidity of the monolayer in the solid phase, whose value has been determined for different lengths of the aliphatic chains.

1. Introduction

Monolayers of amphiphilic molecules spread at the air–water interface exhibit a richness of behaviour attributed to the possibility of both in-plane positional and orientational order as well as conformational order of the hydrophobic tails [1]. Breaks in their isotherms $(\Pi, \mathcal{A})$, where Π is the surface pressure and $\mathcal{A}$ the area per molecule, were long ago interpreted as two-dimensional phase transitions. From a gaseous-like state, condensation can occur to the so-called liquid-expanded (LE) and then to the more condensed liquid-condensed (LC) phase, or directly to the LC phase at temperatures under that of the triple point. On further compression, a solid-like phase is attained.

The question of the nature of these transitions has been most controversial. Concerning the LE–LC transition, Pallas and Pethica [2] have shown that, using carefully purified materials, one does obtain flat isotherms, and it is now well recognized that this transition is of first order. The LC–S phase transition has been studied by grazing incidence x-ray diffraction [3], and its nature could depend on the compound.

Many of the recent experimental advances have been obtained using non-perturbative optical methods allowing the determination of the in-plane structure of the film. Complementing these methods, x-ray reflectivity provides direct and accurate information on the electron density along the normal to the interface, allowing a separation between structural parameters and surface roughness.

2. Experimental procedure

2.1. X-ray reflectivity

The diffractometer for liquids has been described elsewhere [4]. The divergence is low (0.23 mrad), and we used a 50 μm $\times$ 10 mm analysis slit and a copper anode tube. The shape of the reflectivity curves results from interferences between the beams reflected by large electron density gradients, and one obtains information on the projection of the electron surface density $\rho(z)$ onto the normal to the surface. As in many cases, the present system can be described as being composed of chemically homogeneous layers, and a model can be constructed by considering these layers as slabs of constant density. Then, one can take into account the standard deviation of the interface height (its roughness) $\langle \zeta^2 \rangle^{1/2}$, by smearing the model through a convolution with a Gaussian function. In the case of the surface of liquids, the roughness is due to thermally excited capillary waves [5, 6]

$$\langle \zeta^2 \rangle^{1/2} \approx \sqrt{\frac{k_B T}{4\pi\gamma} \left(\log \frac{\gamma}{K \delta q^2} - (\gamma_E + \log 2) \right)^{1/2}} \tag{1}$$

where K is the bending rigidity modulus, γ the surface tension, and γ_E Euler's constant. $\sqrt{\gamma/\kappa} \gtrsim 10^{10}$ m^{-1} is the cut-off at high wavevectors; $\delta q \approx 10^5$ m^{-1}, the diffractometer resolution parallel to the plane of incidence, provides the cut-off at low wavevectors, and one obtains the mean characteristics of the surface over distances up to 1 μm.

2.2. Langmuir trough

Our Langmuir trough [4], made of Teflon, has been designed to allow grazing incidence x-ray experiments. Most important in this case is to adjust the surface level; this is achieved by displacement of a reservoir to within ± 5 μm. The surface pressure (measured with a filter-paper Wilhelmy balance) can be used to control the area. In order to avoid surface vibrations, the liquid layer was only 3 mm deep and the experiments ($\approx$ 12 h duration) were carried out overnight. The trough can be temperature controlled to within ± 0.2 °C. It is enclosed in a box 2 cm high that is kept at saturated humidity, and the whole system is maintained under a slow nitrogen flow.

2.3. Chemicals used

For this study of monolayers on water, we concentrated on fatty acids, rather than on more complex ionized species. Dodecanoic acid (behenic acid) $CH_3(CH_2)_{20}COOH$, and triacontanoic acid (melissic acid) $CH_3(CH_2)_{28}COOH$ were purchased from Fluka (purissimum, for gas chromatography), and these were used as supplied.

Hexadecanoic acid (palmitic acid) $CH_3(CH_2)_{14}COOH$ allows the investigation of both the LC phase at room temperature and the LE–LC transition at a higher temperature since the triple-point temperature is 29 °C [2]. This sample (Fluka, purissimimum, 99.9% minimum, for gas chromatography) was further purified by slow recrystallization from a hexane solution. The spreading solution was prepared by selecting a monocrystal, as checked by x-ray diffraction, then dissolved in n-hexane ($\sim$ 5 mg ml^{-1}). The n-hexane was purified by distillation.

Both the LE, LC and solid phases can be studied using L-α-dipalmitoyl-phosphatidylcholine (DPPC) at room temperature. L-α-DPPC from Sigma was used as supplied.

The pH of the subphase (ultra-pure water from a millipore system) was adjusted to pH 2 using concentrated HCl.

3. Results and discussion

A first step when studying monolayers is carefully to characterize the bare water surface [5,6]. The corresponding reflectivity curve (normalized to $R_F(\theta)$) shown in figure 1 has a Gaussian shape, and the roughness is $\langle \zeta^2 \rangle^{1/2} \approx 0.31$ nm. It should be pointed out that this roughness can be exactly obtained from the coupling mode model for capillary waves ($K \approx 0.12k_BT$ [7]) and equation (1) without any adjustable parameters, indicating that this model provides a 'good' cut-off at high wavevectors.
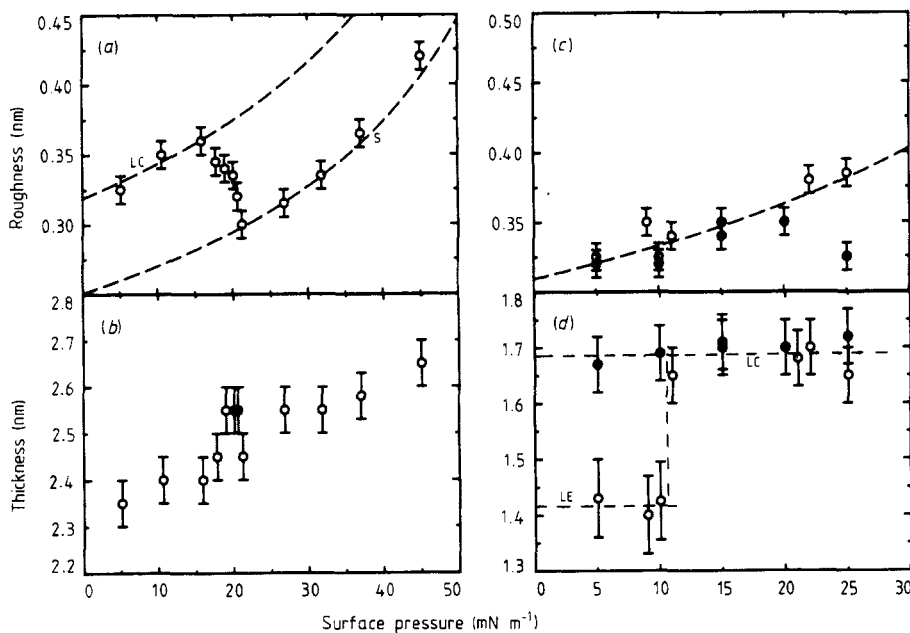

Figure 1. (*a*) Roughness and (*b*) thickness of the behenic acid film; (*c*) roughness and (*d*) thickness of the palmitic acid film. Open circles, $T = 33$ °C; full circles, $T = 22$ °C.

3.1. Structural changes at the LE–LC transition

The results concerning the LE–LC phase transition for hexadecanoic acid at $T = 33$ °C have been detailed elsewhere [8]. Let us just recall that this transition is evidenced by an abrupt increase of the aliphatic medium thickness from 1.42 ± 0.07 nm to 1.68 ± 0.05 nm (figure 1(*d*)), occurring at constant density ($\rho_c = (0.95 \pm 0.03)\rho_{H_2O}$). The constancy of the chain density within the LE and LC phases is a general feature

of Langmuir monolayers. Below the triple point (at $T = 22$ °C), there is no LE phase, and, on compression, the LC phase is directly attained from the gaseous-like state. The characteristics of the layer in the LC phase in this case are identical to those found in the same phase above the triple point, even at pressures for which the LE phase is present at $T = 33$ °C. The results for L-α-DPPC are similar to those obtained with palmitic acid.

The increase in the film thickness can be either viewed as a modification of the molecular tilt angle, or as involving a more subtle conformational change. It is generally admitted that rigid rods cannot exhibit a LE–LC transition [1], and this is consistent with our results, since a reorientation of rigid rods with a mean thickness increasing from 1.42 nm to 1.7 nm would imply an increase of the surface density. On the other hand, the increase in thickness exactly compensates for the diminution of the available area under the constraint of constant density. Indeed, a molecular reorientation has been observed at this transition [9], but it concerns the C—OH bond. This is also consistent with our results (on DPPC). These results suggest that in the LE phase, each chain has one or two gauche conformations, whereas in the LC phase, the molecules have less conformational defects and are tilted (by approximately 30° in our case).
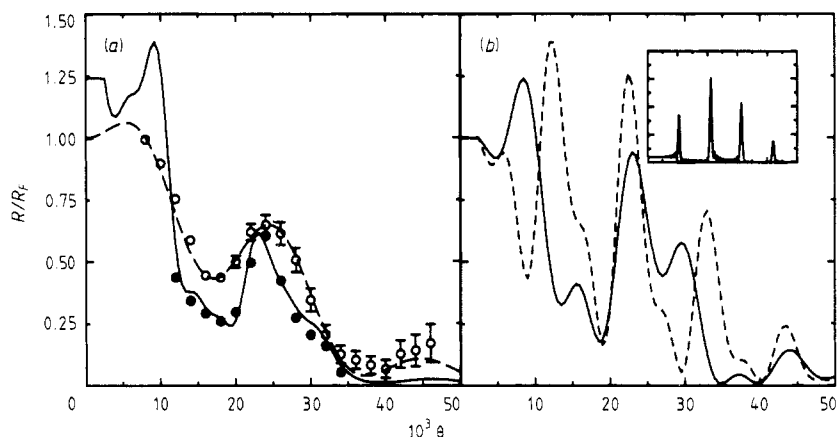

Figure 2. (*a*) Reflectivity curves for melissic acid recorded at different surface pressures: open circles, $\Pi = 20$ mN m^{-1}; full circles, $\Pi = 37.5$ mN m^{-1}. (*b*) Calculated reflectivity curves for multilayers of melissic acid: full curve, three layers; broken curve, four layers; inset, 21 layers.

3.2. Other structural features

Obviously, the curve recorded at 37.5 mN m^{-1} shown in figure 2(*a*) cannot be fitted using the model described above. The peak at 24 mrad is sharper and more intense than expected, and its shape is different. Actually, one can give account of these features assuming that a part of the surface is covered with more than a monolayer. Clearly, an even number of layers (i.e. with polar groups on the top of the stacking) would cause a resulting shape of the reflectivity curve that would be inconsistent with the experimental data (figure 2(*b*)). Moreover, since the particular shape obtained for

an odd number of layers is due to the presence of only an additional layer, it tends to a classical Bragg pattern for an increasing number of layers. This indicates that the monolayer is in equilibrium with multilayers composed of a small (mainly three or five) odd number of layers. This transition to a multilayer can be related to the plateaux which are apparent on a few $(\Pi, \mathcal{A})$ curves, at pressures well below the limit of stability of the film, and corresponds to what is expected to be the mechanism of collapse of monolayers.

3.3. Bending rigidity in the solid phase

Equation (1) gives the principle of our determination of bending rigidity. The roughness of the film is measured for a given resolution of the diffractometer; if the surface pressure is determined otherwise (Wilhelmy balance), K can be calculated. This is, to our knowledge, the only method allowing the measurement of the high bending rigidity modulus of Langmuir monolayers.

3.3.1. Behenic acid (figure 1(a) and 1(b)).
In the LC phase, the roughness increases upon compression as $1/\sqrt{\gamma}$, extrapolating to the roughness of water at $\Pi = 0$. More precisely, the measured roughness follows equation (1), and thus the sole contribution of capillary waves without additional rigidity accounts for it.

A most striking feature is the drop in the roughness at the LC–S transition ($\Pi = 20$ mN m^{-1}). This drop in the roughness is due to the appearance of a high bending rigidity in the solid phase [6], which can be estimated using equation (1), $K = 190 k_B T$. Since K appears in this equation with a logarithmic dependence, the experimental data are consistent with a rather wide range of bending rigidity values, between $120 k_B T$ and $300 k_B T$. After the transition, the roughness again increases as $1/\sqrt{\gamma}$, and thus K remains constant within experimental error.

3.3.2. Other compounds.
The variations of the roughness are similar to those obtained with behenic acid. A drop in the roughness at the LC–S transition corresponds to the appearance of a bending rigidity $K = 15 k_B T$ (with $4 k_B T \leq K \leq 70 k_B T$) for L-$\alpha$-DPPC, and $300 k_B T \leq K \leq 1000 k_B T$ for melissic acid. For hexadecanoic acid (figure 1(c)), at $T = 22$ °C, a significantly lower roughness was obtained at the limit of stability of the layer, corresponding to a bending rigidity $K = 70 k_B T$.

3.4. Simple evaluation of the bending rigidity

This problem, related to that of lipid chain conformation, has only been tackled recently [10]. Due to the very low compressibility (or constant density constraint), any fluctuation of the interface implies conformational changes of the chains. If one only retains the entropic contribution to the Helmholtz free energy F, one obtains

$$K = \left(\frac{15}{4}\right) k_B T \left(\frac{\mathcal{N} L^2}{C_\infty a^2 \Phi^2 \mathcal{A}^3}\right) \propto \mathcal{N}^3$$

where $\mathcal{N}$ is the number of monomers, and the characteristic ratio $C_\infty \approx 6$ [10] has been introduced to take into account the short-range monomer–monomer interactions. The thickness dependence of K is similar to that for a macroscopic plate. With the density of monomers $\Phi = 38.1$ monomomer nm^{-3}, $\mathcal{A} = 0.21$ nm^2/chain, the monomer–monomer distance $a = 0.154$ nm, one gets $K \approx 60 k_B T$ for hexadecanoic acid or L-α-DPPC ($\mathcal{N} = 15$), $K = 200 k_B T$ for behenic acid ($\mathcal{N} = 21$), and $K = 530 k_B T$ for

melissic acid ($\mathcal{N} = 29$). These values account for our experimental data, which are also consistent with values of K obtained for multilayer membranes by other experimental methods [11].

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